CAYLEY, DOROTHY M.

SOME OBSERVATIONS ON THE LIFE-HISTORY OF NECTRIA GALLIGENA, BRES.

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Some Observations on the Life-history of Nectria galligena, Bres.

BY

DOROTHY M. CAYLEY,

Research Student, John Innes Horticultural Institution, Merton, Surrey.

With Plates IV and V.

INTRODUCTION.

THE present investigation was started with the object of finding, if possible, the most vulnerable stage in the life-history of *N. galligena* at which fungicides could best be applied to prevent the spread of the disease. This involved a complete study of the life-history of the fungus itself, and more especially the transition from the summer or conidial stage to the resting or perithecial stage. During the course of this part of the investigation a few hitherto unrecorded observations were made, which were thought to be of sufficient interest to allow of publication.

The description of the life-history is necessarily incomplete, owing to technical difficulties. The fungus is very small and difficult to deal with. The fruiting stages occur on dead or dying bark, and it was found impossible to fix and cut serial microtome sections thin enough to allow of accurate microscopical investigation. It was only after a medium had been elaborated, on which the fungus would complete all the known stages in its life-history in pure culture, that further progress could be made. The preparations on bark, however, served as controls for comparison with the growth in pure culture on artificial media, and the development of the life-cycle proved to be very much the same in both cases.

The fungus is a common parasite on apple and pear trees, giving rise to one of several forms of canker which occur on the stems and branches.

Nectria, under the name of *N. ditissima*, has been described by various workers on a number of other host plants besides apple and pear, such as copper beech, oak, hazel, ash, lime, and others; but it is questionable if these different investigators were really dealing with one and the same fungus. *Nectria* on beech was first described by Tulasne (31), in 1865, under the name of *N. ditissima*, and later Goethe (14–18) was able to show,

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by cross-inoculations, that copper beech could be infected by conidia of *N. ditissima*, Tul., isolated from canker on apple. He also showed that, although *N. ditissima* was generally considered to be a wound parasite, it was possible to infect uninjured shoots with conidia and ascospores, if the shoots were cut off and left without water. Under these conditions infection took place chiefly through the lenticels.

Since Tulasne, a considerable amount of work has been done on the fungus at various times by many different investigators, the most prominent being Willkomm (35), Hartig (20, 21), Goethe (14–18), Lapine (24), Aderhold (1, 2), Appel (3, 4), Wollenweber (3, 4, 36), Weese (33, 34), and others. Willkomm (35), in 1866, was the first to show that canker on beech trees was due to a fungoid parasite, but unfortunately his discovery led no further, as he did not definitely identify the fungus. He observed the conidial stage on beech and called it *Fusidium Candidum*. Hartig (21), took up the investigation in 1877, and showed that beech canker was mainly due to *N. ditissima*, Tul., although cankerous disorganization of bark could be caused by frost and insect injury. On the other hand, Weese (34), one of the more recent investigators, who has studied the Nectriaceae from the systematic point of view, is of the opinion that canker in fruit and timber trees is due to *N. galligena*, Bres., and not *N. ditissima*, Tul. (syn. *N. coccinea*), as held to be the cause by Hartig (20, 21), Goethe (14–18), and Aderhold (2), and that the two fungi are biologically and morphologically distinct. *Nectria galligena* causes definite cankers, whereas *N. ditissima*, Tul. (syn. *N. coccinea*), only breaks out of the bark and does not give rise to cankers. Weese (34) considers the most important characteristics which determine the variety are the formation of the perithecial wall and spore differences; that the presence of a stroma or subiculum is not of such systematic importance as was formerly held, and that one and the same fungus can occur, with or without a stroma, on the same piece of bark. Variations as to the presence or absence of stroma in Hypocreaceae have also been noted by Theissen (30), v. Hoehnel (23), and Wollenweber (36).

It is the opinion of the present author that the *Nectria* with which those workers were dealing—Hartig, Goethe, Aderhold, and others, who proved by inoculations that it gave rise to definite cankers on apple, beech, and other trees, and which they described under the name of *N. ditissima*, Tul.—must have been *N. galligena*, Bres., and that the slight differences between the two fungi were probably overlooked. Goethe had doubts as to whether the fungus with which he was concerned was really *N. ditissima*.

The fungus described in this communication was obtained from canker on apple, and agrees with the description of *N. galligena* given by Weese (33), except that the ascospores, obtained from dehiscing perithecia, and measured

fresh, proved to be rather larger than the dimensions given by Weese. *Nectria ditissima* has shorter ascospores.

Hartig (21) describes the three main stages in the life-history, namely, the microspore, the macrospore, and the perithecial stages, and also found, as did Tulasne (31), spermagonia and spermatia on beech. The best early description of the fungus is given by Goethe (16), 1880, but both he and Lapine (24), 1892, failed to find spermagonia on apple, in spite of careful search. During the present investigation spermagonia or pycnidia were observed occasionally on apple bark affected with *Nectria*, but so far there is no conclusive evidence that the pycnidia observed belonged to *N. galligena*. This point will be more fully discussed later. Much of the work of the later investigators deals with the parasitism of the fungus and the effect of cultural and external conditions on the host and parasite, points which do not come within the scope of this communication.

In *N. galligena*, so far as is known to the author, no investigator has been able to find any definite nuclear fusions, either before the formation of the perithecium or within the tissues of the same. Nuclear fusions have been described within recent years in a number of Ascomycetes, including a few Pyrenomycetes, such as *Gnomonia erythrostoma* (6), *Polystigma rubrum* (5), *Aspergillus herbariorum* (13), &c., and it was thought possible that a more or less reduced stage might occur in *N. galligena*, which so far had been overlooked.

Methods.

Portions of diseased bark and pieces of the medium on which pure cultures were growing were fixed in various fluids, with very variable results. All fixatives containing alcohol, such as acetic alcohol, formal alcohol, 70 per cent. alcohol, absolute alcohol, &c., were found to have a disturbing effect on the nuclei of the ascogonia and had to be abandoned, although the penetration was good.

The only fixative that proved to be of any use for the purpose of the investigation of nuclear detail was Flemming's weaker solution, and the time allowed varied according to the age of the perithecia to be fixed. Perithecia in the youngest stages on artificial media required from 4 to 6 hours' fixation, whereas perithecia on bark and older perithecia on artificial media required longer, and could be left on the fixative 24 hours without harm.

The results were very variable, in spite of the use of the air-pump. This is not surprising considering that tissues of extreme delicacy are enclosed within perithecial walls which vary in toughness according to age. In some cases 1 per cent. urea was added to Flemming's weaker solution to hasten penetration, but the results were disappointing, and latterly Flemming only was used throughout.

Some of the material, especially that on bark, was dehydrated in glycerine and some in alcohol, cleared in chloroform, embedded in 54° C. paraffin, and cut from 2 to 4 μ thick. The youngest stages were cut 2–3 μ.

It was found necessary, except for very young material, to use Henneguy's (22) gelatine-bichromate solution for mounting serial sections to prevent the sections washing off during the process of staining. The solution was prepared as follows: One gramme of gelatine was dissolved in 5,000 c.c. tap-water, to which a trace of bichromate of potash was added before use. The ribbons were placed on the slide, and the gelatine solution—which had been previously warmed in the 54° C. paraffin bath—was pipetted on to the slide. In this way the ribbon expanded rapidly without melting. Sometimes it was found necessary to warm the slide also, with the ribbon still floating on the gelatine solution. The superfluous liquid was then poured off and the slide blotted very gently and placed on several layers of blotting-paper on the top of the paraffin bath, to dry in the light for at least four hours. The bichromate did not seem to interfere with the staining as long as watery stains were used.

Heidenhain's iron-alum-haematoxylin gave by far the best results and was used throughout, counterstained with a saturated solution of lichtgrün or erythrosin in clove oil.

Media.

Conidia and ascospores of *N. galligena* will germinate well on a number of artificial media in common use in the laboratory and will develop considerable mycelial growth, but it was some time before a medium was found on which the fungus would go through all the stages of its life-history. The development is slow, and it takes at least 6–8 weeks, mostly longer, before the perithecial stage appears. Hence a medium had to be devised which would not dry out during that period. Platings proved to be useless on account of this tendency to dry out. Platings were made and the colonies transferred to slopes, or spores from pure culture sown direct on potato, &c.

The only media on which the fungus would develop perithecia were those which contained starch, or some derivative of starch, together with glycerine.

It is to Goethe (16) that we owe the observation that the starch disappears in the starch-containing cells of the cortex when attacked by *Nectria*. It was found that spores sown on potato only gave rise to excessive mycelial and sporodochial growth and reached the macrospore stage, but would develop no farther; whereas ascospores and conidia grown on potato + 1 per cent. glycerine gave rather less mycelial growth and developed perithecia within two months.

Other media containing starch or derivatives of starch were tried with and without glycerine, such as acid pea-agar and acid haricot bean-agar (0·1 per cent. N. HCl), prune juice, oatmeal-agar, &c., with the same result.

The agar-glycerine media were the first to be tried, but these cultures had to be fixed and cut before the asci had developed, on account of the drying out of the medium, and the central cells of the perithecium, which will be more fully described later, showed all the characteristics of normal young perithecia on bark. After these initial trials the potato + 1 per cent. glycerine medium was used throughout the rest of the investigation, as it was found to give the most satisfactory results. Ascospores from freshly gathered dehiscing perithecia on bark in the spring of 1918 completed two life-cycles from ascospore to ascospore during the summer months on the potato-glycerine medium.

The potato slopes were prepared as follows: A wad of absorbent cotton-wool was placed at the bottom of the test-tube and the potato on the wad. A sufficient quantity of 1 per cent. glycerine solution in distilled water was poured into the tube so as to completely cover the whole of the potato, then the tubes were autoclaved and stored until required. Before inoculation the superfluous glycerine solution was poured off. In this way there remained sufficient moisture to last throughout the period of development, if taken out of the incubator after the first two or three days. What effect the glycerine has on the development of the fungus is not known.

After inoculation, the culture tubes were placed in the incubator and kept at a temperature of 25° C. for the first few days, and then left on a shelf in the laboratory, where they were exposed to direct sunlight until 11–12 o'clock in the day during the summer months, in order to give them, as far as sunlight was concerned, approximately the same conditions as the tree from which the fungus had been obtained. This treatment proved highly satisfactory. It was then thought that perhaps a little mechanical pressure might hasten the development of perithecia, as, in the host plant, the mycelium of the fungus is very densely coiled and develops in considerable quantity before the bark is ruptured. Some of the cultures were pressed against the sides of the test-tubes with a sterile glass rod. The fungus, however, developed so well without the pressure, and the different cultures varied as to the amount of mycelial development, probably owing to the small differences in moisture content, size, and other properties of the potato slopes, that it was not possible to come to any definite conclusion on this point without further work under much more stringent standard conditions. During the process of elaborating a suitable medium, the possibility of a symbiotic relationship between a bacterium and *N. galligena* had to be considered, more especially as the microtome preparations of *Nectria* growing on bark frequently showed the presence of a rod-shaped

bacterium in the sporodochium of the fungus. Brzezinski (8) published a paper in 1903 in which he stated that apple canker was a bacterium and not a *Nectria*. This was contradicted by Aderhold (2). The present writer also found that typical sunken cankered areas could be induced in young one-year-old twigs of apple in the open, when inoculated in spring with macrospores obtained from a pure culture. Other inoculations with bacteria isolated from platings of macrospores from bark were tried at the same time, but the wounds healed up naturally and no ill effects could be seen. The inoculated portions of the twigs were enclosed, in both cases, in sterile glass cylinders plugged at each end with cotton-wool to prevent risk of infection from other sources. Various bacterial growths from platings of macrospores were also tried with pure cultures of *N. galligena*. The cocci were discarded and only rod-shaped bacilli used. Tubes infected with spores of the fungus and bacilli at one and the same time showed that the bacilli developed rapidly at the expense of the fungus. Further work on these lines was, however, discontinued when it was found that *N. galligena* would develop perithecia in pure culture on media containing glycerine.

Life-history.

The genus *Nectria* is placed by Saccardo in the family Hypocreaceae, a sub-group of the Pyrenomycetes.

It has a complicated life-history which may be divided roughly into three distinct stages, which occur in the following sequence:

1. The microspore stage, with minute elongate oval hyaline spores, 5–7 μ × 1–2 μ, abstricted from fine hyaline mycelium, sparsely septate and inclined to monopodial branching (Fig. 7).

2. The macrospore stage, with 6–7 septate curved spores, 65–75 μ × 4–5 μ, abstricted from branched conidiophores on a sorus or sporodochium consisting of coarse, densely intertwined, thick-walled mycelium, brownish red in colour when mature (Figs. 1, 4, 10*a*, 11, 12, 12*a*, 13, 13*a*, 13*b*, 24).

3. The perithecial stage, which occurs on the same sporodochium as the macrospores. The red, flask-shaped perithecia contain paraphyses and numerous eight-spored asci. The ostiolum is raised, rather darker in colour than the rest of the perithecium, and is furnished with periphyses. The ascospores emerge through the ostiolum in the form of whity-buff tendrils. The ascospores, 15–21 μ × 6–8·5 μ, are two-celled, with roughened walls slightly tinged with yellow (Figs. 2, 3, 5, 9, 14, 15, 16, 18–22).

All these stages have been described by previous workers, but during the present investigation two-celled multinucleate spores have also been observed both in pure culture and in preparations on bark (Fig. 8). Unstained, these could not be distinguished from two-celled macrospores, hence their function, if any, could not be traced.

Development on Artificial Media.

Microspore Stage.

All stages in the life-history of *N. galligena*, as described by Hartig and Goethe under the name of *N. ditissima*, were observed to occur in pure culture. Ascospores, obtained from tendrils of dehiscing perithecia on apple bark, sown on potato + 1 per cent. glycerine, give rise in the course of a few days to a weft of fine, spreading, hyaline mycelium. This mycelium is sparsely septate, and the branching more or less monopodial. Unicellular microspores, 5–7 μ × 1–1·5 μ, are abstricted from the tips of the fine hyphal branches, and not from differentiated conidiophores. The function of these microspores is not known.

As the fungus colony grows, coarser thicker-walled mycelium develops which is much more septate than the above. The cell-walls are tinged with yellow and the cells contain numerous oil-drops. Branched conidiophores (Fig. 11) arising from a sporodochium of closely interwoven hyphae give off enormous numbers of septate macroconidia, the *Fusarium Willkommii* stage of Weese (34). In the host plant this stage occurs during the months of September and October, or even later if the weather is open (Fig. 1). The macroconidia when fully developed are hyaline, slightly curved with rounded ends, 5–7 septate, 65–75 μ × 5–5·5 μ (Fig. 10), and each cell is uninucleate at first. These macrospores can be easily distinguished from the microspores. They are considerably larger, although all stages from the unicellular to the multicellular macrospores have been observed both on artificial media and on apple bark. The first-formed macrospores on a young sporodochium as a rule have fewer septae.

Macrospores grown in a hanging drop germinate mostly from the terminal cells at either end of the spore (Fig. 13 *b*). The middle cells, under moist conditions, do not germinate very readily. Cultures made from macrospores give rise, in their turn, to microspores and follow the same sequence of stages as cultures made from ascospores. This is the case when macrospores drop away from the sporodochium. But under certain conditions, when undisturbed, as is mostly the case on artificial media, and also in the open in calm autumnal weather, a considerable number of macrospores remain *in situ* and anastomose by throwing out connexions from their central and even their terminal cells (Fig. 13). Only one cell may link up with another cell of a neighbouring spore, or several cells of one spore may be linked with cells of a neighbouring spore or spores. The result is the formation of a palisade pseudo-tissue formed by linked macrospores. This linking is of very common occurrence both on bark and artificial media. This palisade tissue helps to increase the bulk of the sporodochium. At this stage the behaviour of the nuclei of the macrospores is interesting but little understood. The passage of the nucleus from the

cell of one spore to that of another has never been observed, but frequently one cell is seen to contain two nuclei, and the cell to which it is linked is enucleate (Fig. 10 *b*). Also, one or all the cells of a macrospore, when mature, have often been seen to contain two nuclei, but this was thought to be due to the division of the primary nucleus (Figs. 10 *a* and 12 *a*). Macrospores which are quite free and unlinked can have binucleate cells (Figs. 10 *a* and 12 *a*). It is not easy to suggest what this peculiar behaviour indicates. At first it was thought that in *Nectria*, since the perithecia arise on the same sporodochium as the summer macrospores, this fusion of macrospores might possibly be a sexual fusion from which the perithecia eventually arise. There is, however, no proof of this supposition. The fungus is so small, and the mycelium of the sporodochium so twisted and dense, that in spite of prolonged search it was found impossible to trace the origin of the mycelium or cells from which the perithecium arises, or to find any definite connexion except one of position.

Variation in size and shape of the mature macrospores has been described and figured by previous workers in *Nectria* and other forms of *Fusarium* (Fig. 13 *a*). One or more cells in the middle of the spore can become enlarged, with thickened walls and deeply staining rich cell contents; also bodies were observed by the author among the macrospores on a well-developed sporodochium, consisting of a stalk cell and three to four enlarged circular cells, the terminal or penultimate cell of which contained rich cell contents. These bodies certainly did not give the impression of being degeneration forms, but it is impossible to say whether they perform any special function. Lapine thought the enlarged central cells of the macrospore to be of the nature of chlamydospores, but Aderhold, on the other hand, considered them to represent degeneration stages.

Perithecial Stage.

The perithecium arises from a tangle of coiled hyphae (Fig. 23), which appear to branch off from the thick-walled sporodochial mycelium. No differentiated archicarp has ever been observed to which the perithecium could be traced. The sporodochium forms a thick and complex layer of twisted hyphae with walls of varying thickness, and it is not possible to tell which particular knot of hyphae will eventually develop into a fertile perithecium. Sterile perithecia-like bodies occur both on bark and artificial media; they consist of the usual outer layers of brown, thick-walled, deeply staining mycelium, enclosing the thinner-walled hyphae with uninucleate cells. The nuclei of these central cells soon disappear and no further development occurs except increase in size. Schaffnit (27) observed similar bodies in *Fusarium nivale* and considered them to be sclerotial organs. Not infrequently, however, one or more small fertile perithecia have been

observed developing in the internal tissues of the sterile perithecium, more generally near the upper part of the periphery. The rest of the sterile perithecium serves to increase the bulk of the sporodochium ; thus sections of sporodochia in pure culture often show layers of perithecia with walls of varying thickness. The fertile perithecium originates in the same way, and consists of the thick-walled outer layers and the thin-walled internal tissues with uninucleate cells. The nuclei of these cells are distinct and homogeneous and stain readily with the usual nuclear stains. Some of these cells eventually become absorbed, while others appear to divide and form several layers of delicate tissue immediately beneath the perithecial wall. On potato-glycerine the growth of the sporodochium is much looser, hence the knots of hyphae which give rise to perithecia can be more easily distinguished. The appearance of the fungus, however, in no way tends to show that the conditions are not conducive to normal development. In the earliest stages, one or more cells in these knots are larger and sometimes binucleate (Fig. 23), but in such rapidly growing tissue this binucleate condition is probably due to division of the primary nucleus prior to cell division. The development of the first stages of the perithecium is rapid, and difficult to follow. Before any disintegration of the central cells of the perithecium occurs, the ascogonia begin to develop (Figs. 15, 16, 19–22), and are easily distinguishable from the surrounding tissues, owing to their larger cells and denser cell contents. They are coiled multicellular structures with multinucleate cells, and the nuclei show a definite nucleolus and nuclear area. There is more than one ascogonium in the perithecium. In two instances three ascogonia could be counted with a fair degree of certainty, but the perithecia are so small at this stage, and the ascogonia so much intertwined, that it is difficult to trace each ascogonium throughout its length through a series of sections, and to determine whether the number of ascogonia varies in different perithecia.

The ascogonia originate from thin-walled tissue at the base of the perithecium, and appear to push their way up amongst the central cells (Fig. 15). Some of the latter begin to disintegrate. Fig. 15 shows the bases of two young ascogonia in a young perithecium, and disintegrating central cells. It was not possible to determine whether the ascogonium begins as one cell and becomes multicellular by growth and cell division, or whether it is a multicellular structure from the first, being gradually differentiated from a row of central cells. No case of an incipient unicellular ascogonium was observed, but the examination of a considerable number of young perithecia in different stages led to the conclusion that the multicellular condition was most probably due to growth and cell division of a differentiated primary cell. The cells of the ascogonia increase in number as the perithecium develops, until a stage is reached in which the interior of the perithecium is filled with a considerable number

of large multinucleate cells (Fig. 5). Portions of the ascogonia appear to be narrower and stain more deeply than others, but whether these portions are of the nature of trichogynes, or whether they are the portions which are growing the most actively, could not be determined. No nuclear fusions were observed in the ascogonia, but the nuclei tend to associate in pairs as the ascogonial cells mature (Fig. 5). In one instance only (Fig. 22) there were indications of the passage of the nucleus of one cell to another. As will be seen in the figure, there is a large and very definite nucleus in one cell and a definite pore in the cell-wall, through which a nucleus of a neighbouring cell is about to pass. It will also be seen that some ascogonial cells are in a more advanced stage of disintegration than others. The culture from which this preparation was made was grown on potato-glycerine from ascospores obtained from dehiscing perithecia on bark, and was thirteen weeks old. Figs. 19, 20, 21, 22, are consecutive sections of the same perithecium cut 3 μ thick.

The ascogonia do not appear to function, in that they do not give rise directly to ascogenous hyphae. Two or more ascogonial nuclei pass into the hyphae which grow out from the ascogonial cells. The ascogonia gradually degenerate and the hyphae in their turn completely fill the cavity of the perithecium. The actual ascogenous hyphae which give rise to asci arise *de novo* at the base of the perithecium, from cells which contain two or more nuclei showing the same characteristics as the nuclei of the ascogonia, namely, they have a well-marked nucleolus and nuclear area, and occasionally show chromatin granules. The origin of these basal cells could not be traced. The nuclei of these cells are of two sizes, large and small, as seen in Fig. 18, but it was not possible to determine whether the larger were the result of the fusion of two smaller nuclei. The smaller nuclei tend to associate in pairs at this stage also. The perithecium is completely filled with paraphyses before the asci begin to develop. In spite of careful search, no crosier formation or further nuclear fusions could be found prior to the development of the ascus, and the study of the nuclear divisions in the ascus had to be abandoned owing to difficulties in technique. All fixatives so far used for the purpose of fixing perithecia at the stage at which the asci develop either failed to penetrate the hard outer perithecial wall, or, if the penetration was satisfactory, microtome sections could not be cut sufficiently thin. The large number of paraphyses present and the extreme smallness of the whole structure made accurate observations impossible.

The ovoid perithecia contain numerous asci, which can be seen in all stages of development in the perithecium. The asci contain eight two-celled ascospores with thick, slightly roughened walls (Figs. 9, 14). The ascospores emerge as yellowish-white tendrils through the raised ostiolum at the apex of the perithecium. On the host plant perithecia begin to form in the late

autumn, develop slowly throughout the winter months, and dehisce in spring. Material gathered in February showed perithecia in all stages of development; in April the perithecia are mostly fully grown and about to dehisce.

Pycnidia.

With regard to the vexed question as to the occurrence of pycnidia in the life-history of *N. galligena*, pycnidia have been seen from time to time in preparations of the fungus growing on bark. They occur as a rule in close proximity to the perithecia, and appear to originate from the same sporodochium (Fig. 16). So far, no mature pycnidia containing spores have been observed in any of the pure cultures on artificial media. On bark, the pycnidial wall consists of one or two fairly regular layers of thick-walled cells (Fig. 17), whereas the young perithecial wall contains more than two outer layers, is much more irregular, and the transition from the outer layers to the thin-walled central cells is more gradual than is the case in the young pycnidium. Also the internal tissues of the young perithecium vary considerably in size of cell and capacity for taking up stains, as compared with the central portions of the pycnidium, which stain much more uniformly. It is, however, somewhat difficult to distinguish between pycnidia and immature perithecia, more especially when the latter are filled with paraphyses and the sections happen to be transverse or oblique. On artificial media, owing to the more irregular growth of the tissues of the sporodochium, and the variability in thickness of the walls of young perithecia, immature pycnidia may possibly have been overlooked. One or two doubtful cases occurred, but it was impossible to arrive at any definite conclusion. If pycnidia do occur in the life-history of *Nectria galligena*, of which there is not sufficient proof, they are probably abortive, as the fungus will complete its life-cycle from ascospore to ascospore on artificial media without the development of pycnidia. The unicellular pycnospores (Fig. 17) are abstricted from simple unbranched sporophores in acropetal succession.

Two-celled Multinucleate Spores.

At the stage in which the young perithecia begin to develop, two-celled multinucleate spores (Fig. 8) have been observed both on bark and artificial media. Eight or more nuclei occur in each cell, when the spore is mature. Unstained, they cannot be distinguished from two-celled macrospores, and no signs of germination could be seen in stained preparations.

Summary.

The fungus described in this paper was isolated from canker on apple, and agrees in morphological and biological characteristics with *N. galligena*,

Bresadola, described by Weese as causing apple canker, with the exception of the somewhat larger dimensions of the ascospores.

The fungus will complete its life-history on media containing starch or a derivative of starch with 1 per cent. glycerine.

No differentiated archicarp was observed to which the development of the perithecium could be traced; the perithecium arises from a coil of vegetative hyphae in the sporodochium.

Several ascogonia occur in the young perithecium; these degenerate and disappear before the formation of the asci.

The ascogenous hyphae, from which the asci develop, arise *de novo* from cells at the base of the perithecium, the nuclei of which have the same characteristics as the nuclei of the ascogonia.

The further development of the perithecium could not be followed.

Besides the three different kinds of spores known to previous investigators, a fourth form, a two-celled multinucleate spore, was observed.

Pycnidia occur on bark, but no mature pycnidium was seen in preparations of the fungus in pure culture on artificial media.

Except in section, pycnidia on bark cannot be distinguished from young perithecia, and although in some instances they appear to develop on one and the same sporodochium, there is no conclusive evidence that pycnidia occur in the life-history of *N. galligena*.

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EXPLANATION OF PLATES IV AND V.

Illustrating Miss Dorothy M. Cayley's paper on the Life-history of *Nectria galligena*, Bres.

The preparations are from material fixed in Flemming's fluid, and stained with iron-alum-haematoxylin, unless otherwise stated.

PLATE V.

Fig. 1. Summer or macrospore stage on bark. × $2\frac{1}{2}$.

Fig. 2. Winter or perithecial stage on bark. Perithecia beginning to dehisce. × $4\frac{1}{2}$.

Fig. 3. Sporodochium and cluster of young perithecia on potato-glycerine. Fixed and stained.

Fig. 4. Sterilized twig of Cox's Orange apple infected with pure culture of *N. galligena*. Fixed and stained. (*a*) Mycelium pushing up the cuticle. (*b*) Twisted mycelium penetrating the tissues of the cortex. (*c*) Cuticle. (*d*) Intercellular mycelium. (*e*) Intracellular mycelium. (*m*) Young macrospore.

Fig. 5. Young perithecium on bark. Central region filled with ascogonial cells. Note nuclei associating in pairs. Fixed and stained.

Fig. 6. Pure cultures of *N. galligena*. (*a*) Young culture from ascospores on oatmeal-agar + 1 per cent. glycerine, 7 days old. (*b*) Older culture from ascospores on oatmeal-agar + 1 per cent. glycerine. Perithecia beginning to develop. 32 days old. (*c*) Cultures from ascospores on potato-glycerine medium. 32 days old.

PLATE VI.

Fig. 7. Microconidia and monopodial sporophore. × 1000. Fresh material.

Fig. 8. Multinucleate bicellular spore. × 1,000. Fixed and stained.

Fig. 9. Ascospores. × 1,000. Fresh material.

Fig. 10. (*a*) Macrospores. × 1,000. Stained *intra vitam*. (*b*) Large macrospores, showing one cell with two nuclei, with a neighbouring enucleate celled. Fixed and stained.

Fig. 11. Young macrospores on branched sporophores. × 1,000. Fresh material.

Fig. 12. Macrospore giving rise directly to another sporophore and macrospores. × 700. Fresh material. (*a*) Mature macrospore with three binucleate cells. Stained *intra vitam*.

Fig. 13. (*a*) Linked mature ascospores, with cells containing oil drops, forming pseudo-palisade tissue, destined to become sporodochial tissue. × 800. Fresh material grown on oatmeal-agar. (*b*) Various metamorphosed macrospores. Stained *intra vitam*.

Fig. 14. Asci in various stages of development. Fixed and stained.

Fig. 15. Young perithecium, showing bases of two very young ascogonia with multinucleate cells. Grown on potato-glycerine medium. × 1,300. Fixed and stained. Drawn camera lucida.

Fig. 16. Two perithecia, one on either side of a pycnidium, on bark. (*a*) Young perithecium with ascogonia. (*b*) Older perithecium filled with paraphyses before the development of asci. (*c*) Pycnidium. Drawn camera lucida. × 310.

Fig. 17. Portion of the pycnidium in Fig. 16, showing pycno-sporophores abstricting pycnospores in acropetal succession. × 1,500.

Fig. 18. Cells of the hypothecial layer at the base of the perithecium containing large and small nuclei, from which the asci develop. × 1,800. Fixed and stained.

Figs. 19, 20, 21, and 22. Consecutive sections of a young perithecium, cut 3 μ thick, showing multicellular multinucleate ascogonia. One ascogonium is degenerating. Grown on potato-glycerine medium. × 1,600. Fixed and stained; drawn camera lucida.

Fig. 23. Early stage of perithecium, coiled hyphae. Fixed and stained. × 1,000.

Fig. 24. Linked macrospores, showing one cell with two nuclei. Fresh material stained *intra vitam* with sat. erythrosin in glycerine.

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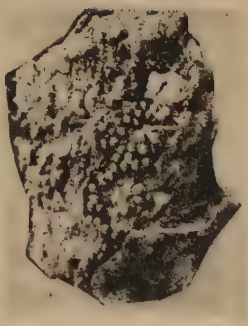

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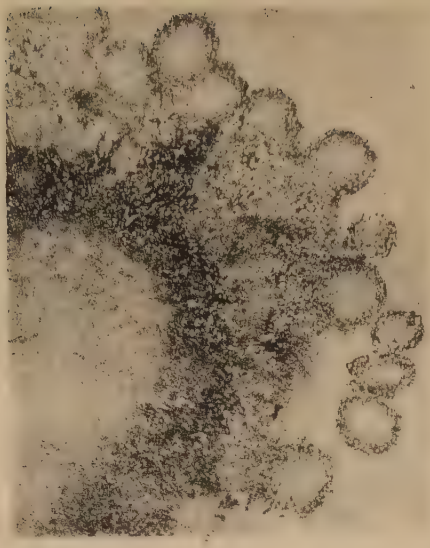

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a b c

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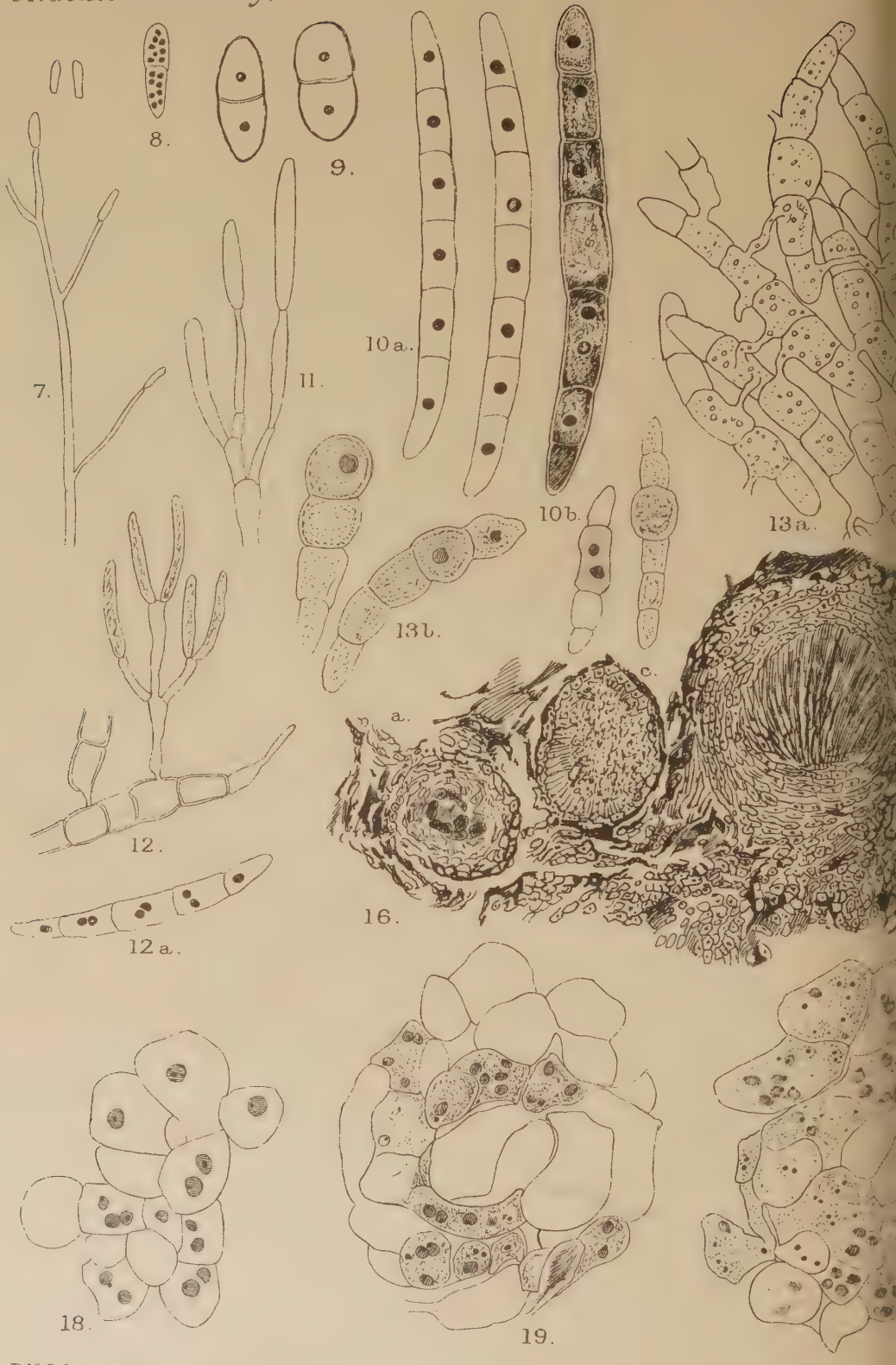

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CAYLEY—NECTRIA GALLIGENA.

13 b.

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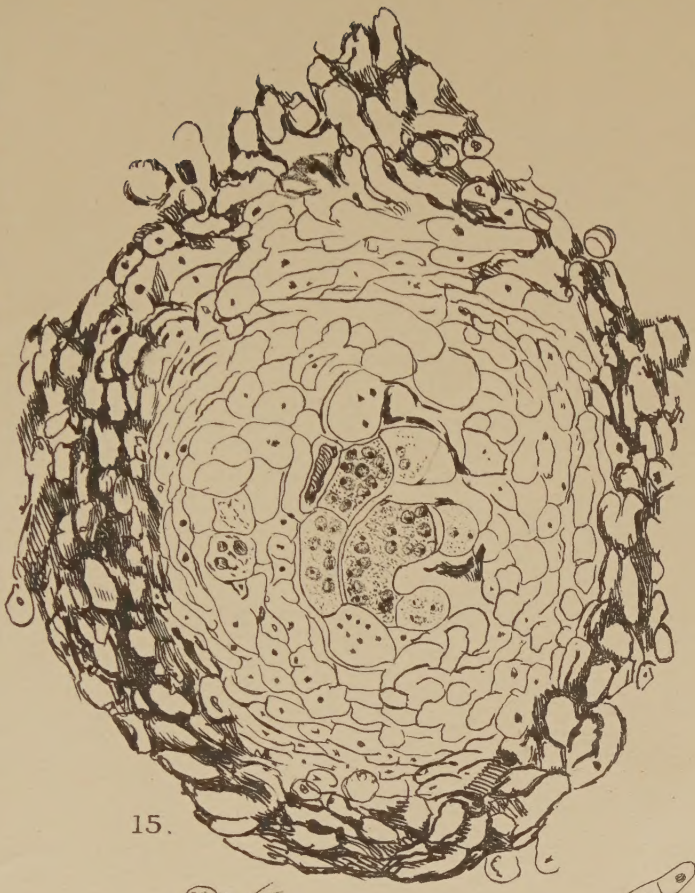

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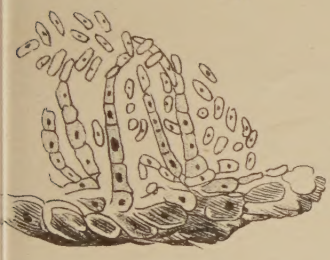

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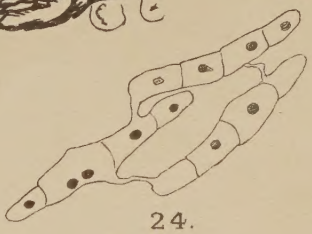

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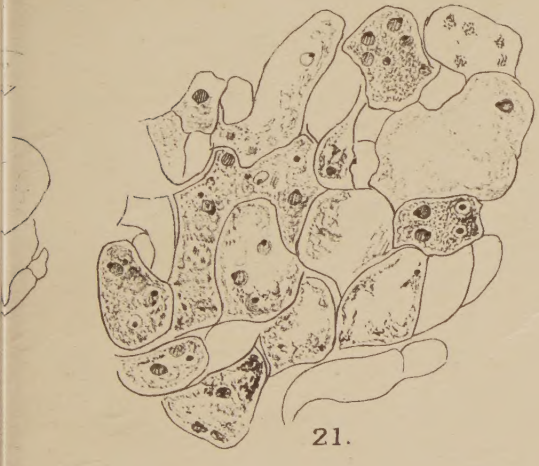

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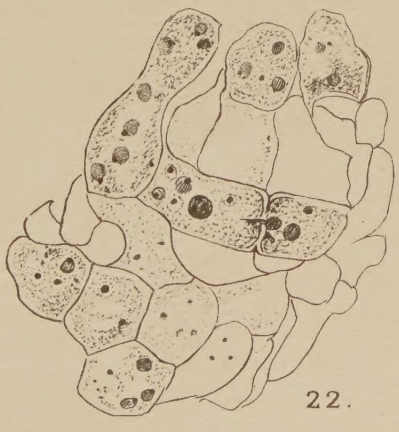

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